54. (Amended) A method according to Claim 49 wherein said FLP recombination target site is introduced into the genome of said **[host]** mammalian cell by transfecting said **[host]** mammalian cell with a DNA fragment containing at least one recombination target site **[therein]**.

55. (Amended) A method according to Claim 49 wherein the DNA of said **[host]** mammalian cell contains at least one FLP recombination target site, and wherein said FLP recombination target site is so positioned that the introduction of additional DNA sequences therein will inactivate the target gene.

Remarks

Of the currently pending claims, claims 25-28 and 42-55 are under consideration. By the present communication, claims 25-28, 42-44, 47-51, 54 and 55 have been amended to define Applicants' invention with greater particularity. No new matter has been introduced by the subject amendments as the amended claim language is fully supported by the specification and original claims.

Applicants gratefully acknowledge the Examiner's thoughtful review of the application and suggestion of amendments to the claims. Wherever possible, Applicants have adopted the suggested language. In addition, the language of the claims has been simplified by relying on the definition of the terms "first DNA," "second DNA," and "genes of interest", as set forth in the specification at pages 12 and 13. In light of these amendments and the following remarks, reconsideration of the rejections set forth in the September 10, 1992, Office Action is respectfully requested.

The invention of the claims under consideration is directed to the site-specific integration of DNA into the genome

of a cell or organism. The specific site of integration is referred to as a "FLP recombination target site" (i.e., an "FRT"). At a minimum, an FRT comprises two 13 base-pair repeats, separated by an 8 base-pair spacer, as follows:

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                    -Spacer-
5'-GAAGTTCCTATTC[TCTAGAAA]GTATAGGAACTTC-3'
                   XbaI
                   site
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The nucleotides in the above "spacer" region can be replaced with any other combination of nucleotides, so long as the two 13 base-pair repeats are separated by 8 nucleotides.

Because the FRT is a relatively short sequence of nucleotides, it can be integrated into the DNA of a host without unduly disrupting the host's normal processes. Once the FRT is integrated at a confirmed site of interest, the predictable integration provided by the FLP/FRT recombination system used in accordance with the claimed methods alleviates the randomness commonly associated with the transfection of DNA.

The objection to the specification, and rejection of claims 25-28 and 42-55 under 35 U.S.C. §112, first paragraph, as allegedly failing to provide a reasonable written description, enablement and best mode for practicing the invention, is respectfully traversed. However, it is apparent from the discussion of the rejection set forth in the Office Action (see paragraph bridging pages 2-3 of the Office Action) that the Examiner has measured the "invention", for purposes of evaluating the specification for compliance with 35 U.S.C. §112, first paragraph, by reference to the Background rather than the specific requirements of the claims. When the specification is properly evaluated in terms of the claimed invention, it is clear that the specification meets the requirements of 35 U.S.C. §112, first paragraph.

The Examiner states (at page 2 of the Office Action, at lines 5-8 of the main paragraph) that "the specification at pages 1-2 creates doubt as it indicates that manipulation is impaired due to inability to control site of integration, number of copies, temporal expression, and the like." The rejection under 35 U.S.C. §112, first paragraph, is then justified based on the alleged failure of the specification to provide an enabling disclosure for site-specific integration of DNA coding for the FLP recombination target site in the host cell, host organism or transgenic animal. However, the claimed invention is not directed to site-specific integration of FLP recombination target sites, but to site-specific integration of DNA into FLP recombination target sites.

The invention as set forth in the amended claims is directed to "precisely targeted *in vitro* integration of DNA into the genome of a non-human host organism," "site-specific integration of transfected DNA in to the genome of a mammalian cell," and "site-specific integration of transfected DNA into the genome of a transgenic non-human mammalian cell." The claims plainly recite "introducing . . . DNA into" or "integration of . . . DNA . . . at" the FLP recombination target site. Accordingly, when properly considered in terms of the claimed invention, the specification clearly meets the requirements of 35 U.S.C. §112, first paragraph.

Applicants appreciate the Examiner's careful consideration of the Background and understanding of the problems in the art posed by random integration of transgenes. However, the claims are the proper measure of the invention, not the background section of the specification. The background section of the specification merely serves to familiarize the reader with the state of the art and frame some of the more prevalent problems faced by those who practice this art. The background does not in any way limit the pending claims.

Nevertheless, identifying the location of a FLP recombination target site in the genome of a non-human host organism is well within the capabilities of one of ordinary skill in the art, especially given the fact that the nucleotide sequence of an FRT is set forth at page 11 of the specification. Techniques for identifying the location of a known sequence in a genome are well known and readily available to those of skill in the art, as evidenced by the references cited by the Examiner as a basis for rejection under 35 U.S.C. §102 and §103.

The rejection of claims 25-28 under 35 U.S.C. §112, first paragraph, as the disclosure is allegedly non-enabling for claims directed to *in vivo* host cells and human host organisms, is respectfully traversed. Although Applicants believe the claims are enabled by the disclosure, the claims have been amended in a manner that renders the rejection moot.

The rejection of claims 25-28 and 42-55 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the invention is respectfully traversed. As amended, the claims are submitted to be in full compliance with 35 U.S.C. § 112, second paragraph.

The rejection of claims 25-28 under 35 U.S.C. §102(b) as allegedly anticipated by Golic et al., Cell 59:499-509 (1989), is respectfully traversed. Golic et al., cannot be anticipatory of the elected claims because it does not disclose every limitation of the claimed invention. The recombination disclosed by Golic et al. is achieved in a wholly different manner than the claimed method of targeted integration.

While the Examiner correctly characterizes the cited reference as disclosing the site-specific recombination of *Drosophila melanogaster* DNA using DNA coding for FLP and FRT,

this characterization does not compensate for the total failure of the reference to disclose any of the steps required by the claimed methods. The reference does not disclose or suggest introducing a FLP recombination target site into the genome of cells which are compatible with the cells of the non-human host cell. Rather, the reference discloses fertilization of a female gamete containing an FRT recombination target site with a male gamete containing an inducible FLP recombinase gene.

The Examiner suggests that one gamete of the parent could be considered a cell compatible with the non-human host organism and that the other gamete could be considered a non-human host organism. However, to do so is a corruption of the English language that prostitutes the unique nature of germ line cells. A gamete is a cell provided by the parent, not the progeny resulting from its fertilization. It is only at the point of fertilization, when the gametes fuse to become a zygote, that a "non-human host organism" could fairly be said to exist. Thus, Golic et al. does not disclose or suggest the introduction of a FLP recombination target site into the genome of cells compatible with the cells of the non-human host organism. Nor does it disclose the introduction of the transformed, compatible cell into the non-human host. Therefore, Golic et al. cannot anticipate the invention as defined by claims 25-28, because the reference does not disclose every limitation of the claimed invention.

The rejection of claims 25-28 and 42-55 under 35 U.S.C. §103 as allegedly being unpatentable over Sauer, U.S. Patent No. 4,959,317 (1990) taken with Golic et al., is respectfully traversed. In this regard, the Examiner states that Sauer discloses site specific recombination of mammalian cells using plasmids with the DNA coding for *Cre* and *lox*. The Examiner acknowledges that Sauer does not disclose the use of DNA coding

for FLP and FRT, but contends that Golic et al. provides the motivation to combine the disclosures of Sauer and Golic et al.

Evidence of the nonobviousness of the invention defined by claims 25-28 and 42-55 is found in the very references cited by the Examiner in support of this rejection. The *Cre/lox* recombination system described by Sauer involves a different protein (having a different structure) than the protein employed by the FLP/FRT recombination system. In addition, the Cre/lox recombination system involves a different recognition sequence than the recognition sequence of the FLP/FRT recombination system. Finally, the Cre/lox recombination system is a prokaryotic recombination system originating from bacteriophage P1. In contrast, the FLP/FRT recombination system employed in the practice of the present invention is an eukaryotic recombination system originating from the 2μ plasmid of *Saccharomyces cerevisiae*. Due to the significant differences in structures, recognition sequences and the differences between eukaryotes and prokaryotes, one of ordinary skill in the art could not expect, with any certainty, that a protein that has evolved to function in an eukaryotic cell would function in the same way as a protein that has evolved to function in a prokaryotic cell.

As set forth above, the method disclosed in Golic et al. is distinct from the methods of claims 25-28 and, contrary to the position asserted by the Examiner, provides no suggestion to combine the disclosure of Golic et al. with the methods disclosed in Sauer. While the authors of Golic et al. may have had an expectation that it would work, to some extent, in some other organisms, nothing suggests that it would work like the *Cre/lox* recombination system disclosed in Sauer. Moreover, the authors' expectation seems to have been speculative at best, as they only "suspect" that other tissue of the very organism that they were studying would be susceptible to FLP-catalyzed recombination

between FRTs. The combination of the Golic et al. and Sauer is, therefore, seen to be advanced only when viewed in hindsight, having benefit of the present disclosure. Such use of Applicants' disclosure is improper.

The further rejection of claims 25-28 under 35 U.S.C. §103 as allegedly being unpatentable over Sauer taken with Golic et al. in further view of Palmiter et al., Ann. Rev. Genet. 20:465-499 (1986), is respectfully traversed. Further reliance on Palmiter et al. is not sufficient to cure the deficiencies of the primary references, as set forth above. Palmiter et al. is, at best, a background reference dealing with methods of gene transfer. The reference does not, however, disclose or suggest the site-specific introduction of DNA into any cell or organism, by any means.

In accordance with 37 CFR 1.97, enclosed are three additional references recently called to Applicants' attention in the PCT counterpart of this application. For the convenience of the Examiner, these references are listed on the attached Form PTO-1449, and a copy of each is enclosed herewith. It is respectfully requested that these references be considered in the examination of this application and their consideration be made of written record in the application file, although none of these additional references are any more relevant than the references already of record.

For the reasons discussed above and in light of the amendments to the claims, Applicants' claimed invention is fully enabled by the specification and is not disclosed or suggested by the prior art. Applicants respectfully request the Examiner withdraw the various rejections set forth in the Office Action and earnestly solicit allowance of claims 25-28 and 42-55. In the event there are minor matters to be resolved in view of this communication, the Examiner is invited to contact the undersigned

at the phone number given below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

3/9/93
Date

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Attachments: Form PTO-1449
Three references